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Does your preservative preserve? A comparison of the efficacy of some pitfall trap solutions in preserving the internal reproductive organs of dung beetles

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Abstract

Eight pitfall trap preservatives, brine, saturated borax solution, propylene glycol (33%, 50%, 75%), white vinegar, 100% ethylene glycol, FAACC (Formaldehyde 4%, Acetic acid 5%, Calcium chloride 1.3%), 4% phosphate buffered formaldehyde and 96% ethanol, were compared for their ability to preserve the internal reproductive organs of the dung beetle *Liatongus militaris* (Laporte) (Scarabaeidae: Scarabaeinae) for up to 28 days in the field. Relative evaporation rates of the preservatives in riparian vine thicket and low open woodland environments were also compared. 96% ethanol, FAACC and 4% phosphate buffered formaldehyde were the only solutions found to preserve all of the internal reproductive organs. Evaporation rates were lower in the vine thicket and some preservatives such as ethanol completely evaporated within seven days. 4% phosphate buffered formaldehyde is recommended as a preservative for pitfall traps left in the field for periods longer than one week. Possible ways to prevent trap interference by mammals and risks to the environment due to overspill are also discussed.

Keywords

Pitfall traps, preservative, killing agent, dung beetles, reproductive organs, ovary, oocytes, formaldehyde, ethylene glycol, propylene glycol, mammal exclusion, evaporation

Introduction

Pitfall traps have long been recognised as an effective and inexpensive technique for sampling arthropods, including Coleoptera (Greenslade and Greenslade 1971; Gist and Crossley 1973; Luff 1975; Newton and Peck 1975; Topping and Sunderland 1992; Weeks and McIntyre 1997). The efficiency of pitfall traps may be influenced by aspects of construction, such as shape, size and materials (Luff 1975; Brennan et al. 1999); spacing and transect design (Luff 1975; Ward et al. 2001; Perner and Schueler 2004; Larsen and Forsyth 2005); and the use of various baits, killing agents, and preservatives (Luff 1968; Greenslade and Greenslade 1971; Weeks and McIntyre 1997; Pekar 2002). Although various killing agents and preservatives are commonly used in pitfall traps, there is a lack of studies testing the efficacy of preservative type in preventing the decomposition of collected samples. This gap in the literature is particularly evident in regards to the deterioration of insects' internal organs (Sasakawa 2007). The examination of internal organs and, in particular, reproductive organs can provide useful ecological information such as the reproductive seasonality and physiological age of insects (Tyndale-Biscoe 1978, 1984; Stork and Paarmann 1992; Tyndale-Biscoe and Walker 1992). The time during which preservatives remain effective is also a largely unknown component of this trapping technique. A probable reason that this duration had not been investigated is that most researchers either service their pitfall traps within a few days of setting or baiting or do not require internal organs of specimens to be preserved.

However, short-term trap servicing is not always feasible and this is especially true for broad scale studies that require trapping to be conducted at many locations or in situations where trapping sites are difficult to access. This results in traps containing captured insects being left *in situ* in preservative for prolonged periods (e.g. up to one month), and presents a serious problem for the study of the internal organs of insects, especially with preservatives prone to evaporation. This makes the choice of a suitable preservative critical. For these reasons, the ideal preservative must: 1) adequately preserve the target organism and its internal organs, and 2) not completely evaporate between consecutive servicing intervals. The aim of this study was to identify a preservative suitable for use in long-term field studies that maintains the internal organs of insects and arthropods for periods of up to one month.

Methods

Specimens and location. This experiment was performed on *Liatongus militaris* (Laporte), an African species of dung beetle introduced to Australia. Individuals were hand collected on 30th October 2006 from dung within a cow paddock behind the James Cook University campus grounds in Townsville, tropical north Queensland, Australia (19°19' S, 146°45'E). This species was chosen because specimens were very abundant and easily obtained.

Preservatives. A total of eight preservatives were tested for their efficacy in preserving the internal organs of *L. militaris*. These preservatives were chosen based on their previous use or recommendation as pitfall trap solutions (see Hall 1991; Weeks and McIntyre 1997; Pekar 2002; Perner and Schueler 2004; Norden et al. 2005; Schmidt et al. 2006; Jud and Schmidt-Entling 2008). Water is also commonly used as a killing agent in pitfall traps but due to its obvious lack of preservative properties it was not tested. The eight preservatives were:

1. Saturated salt solution – NaCl dissolved in hot water until the saturation point was reached (hereafter referred to as brine).
2. Saturated borax solution – Harper's Powdered Borax dissolved in hot water until the saturation point was reached.
3. White vinegar
4. Propylene glycol (33%, 50%, 75%)
5. Ethylene glycol (100%)
6. Ethanol (96%)
7. FAACC (Formaldehyde 4%, Acetic acid 5%, Calcium chloride 1.3%)
8. 4% phosphate buffered formaldehyde (hereafter referred to as 4% PBF)

Forty dung beetles were immediately killed in the field by submersion in each of the eight preservatives, and in the case of propylene glycol in each of the three different concentrations. The dead beetles and 400 ml of preservative were placed into round plastic take away containers (11 cm diameter, 800 ml capacity). These containers were placed in the ground approximately 5 m apart in a riparian vine thicket forest type within the James Cook University campus grounds. Due to the high evaporation rate of 96% ethanol specimens were kept in this preservative within sealed vials in the laboratory. Gardening mesh (3 mm × 3mm gap size) was tied around the opening of the container to prevent other dung beetles, especially *L. militaris*, from entering the preservative at a later date. A plastic roof was placed above the trap to prevent rainfall from diluting the preservative. This ensured that the dung beetles and the preservatives would experience similar environmental conditions to that of a pitfall trap in the field.

Dissections. After one week submerged in the preservatives, ten dung beetles from each preservative were removed and dissected. Since it is common practise to store pitfall catches in ethanol once they have been cleared from the field (regardless of the type of preservative actually used in the pitfall trap) some individuals from propylene glycol, FAACC and 4% PBF were also transferred to 96% ethanol one day before they were dissected in order to test if later transference to ethanol alters the final preservation of specimens. All dung beetles were dissected under water in a wax-lined Petri dish with the visual aid of a stereo microscope. During dissection, preservation of internal organs was noted. The internal organs / structures that were used to test the preservation strength of the preservatives were selected based on their previous use in

physiological age grading studies (Tyndale-Biscoe 1978, 1984). These included the: germarium, ovariole wall, small oocytes, large oocytes, calyx, male follicles / testes, vas deferens, accessory glands, fat bodies and the gut. Internal organ condition was assessed qualitatively. As a result all comparisons were based on observations rather than measurements.

Preservation categories. An overall preservation category was assigned to each preservative. The preservation categories depended on how well a preservative preserved the internal organs of individuals when compared to freshly killed individuals. Preservation of the female ovary was regarded as more important, since most of the characteristics used to identify the various physiological stages of dung beetles are derived from the female ovary. Nonetheless, preservation of other organs including the male reproductive system, fat bodies and gut was also taken into consideration. The four preservation categories were poor, moderate, good and very good and are defined as follows:

Poor – Female reproductive organs not preserved or highly degraded. Size and shape of oocytes cannot be determined. Ovariole base and calyx degraded thus not allowing presence of yellow body (corpus luteum) to be detected. Male reproductive organs may still be detectable but size, shape and colour distorted. Gut lining not preserved leading to the release of gut contents into the abdominal cavity. Head, thorax and abdomen easily detach.

Moderate – Female reproductive organs partially preserved. Oocytes may be preserved but their size, shape and colour cannot be determined. Ovariole base and calyx degraded thus not allowing presence of yellow body to be detected. Male reproductive organs partially preserved but size, shape and colour distorted. Male and female reproductive organs cannot be manipulated without causing irreversible damage. Gut lining not preserved leading to the release of gut contents into the abdominal cavity. Head, thorax and abdomen easily detach.

Good – Female reproductive organs sufficiently preserved. Oocytes retain size, shape and colour. Ovariole base and calyx adequately preserved thus allowing presence of yellow body to be detected. Male reproductive organs preserved retaining their size, shape and colour. Male and female reproductive organs cannot be manipulated without causing irreversible damage. Gut lining preserved retaining gut contents. Head, thorax and abdomen do not easily detach.

Very good – Female reproductive organs ideally preserved. Oocytes retain size, shape and colour. Ovariole base and calyx adequately preserved thus allowing presence of yellow body to be detected. Male reproductive organs preserved retaining their size, shape and colour. Male and female reproductive organs can be freely manipulated without causing irreversible damage. Gut lining preserved retaining gut contents. Head, thorax and abdomen do not easily detach.

If the category was poor or moderate after the first seven days then no more individuals were dissected from that specific preservative since further submergence would have not increased the preservation quality of the specimens. If the category was good

or very good then the remaining specimens were left in the preservative and ten more individuals were dissected after seven more days. This continued up until 28 days.

Evaporation rates. Four pitfall traps containing 400 ml of each of the above preservatives/propylene glycol concentrations and water (control) were placed in the ground approximately 5 m apart in both low open woodland and riparian vine thicket forest types within the James Cook University campus grounds during a spring period (7th September until 10th October 2007). The two forest types provided different levels of shading at the ground surface (hence different environmental conditions) allowing evaporation rates of the preservatives to be compared in response to contrasting habitat conditions. Since the low open woodland forest had an open canopy pitfall traps placed here experienced mostly sunny and dry conditions. The closed canopy riparian forest was maintained by the university gardeners by daily misting of the area. Pitfall traps placed here experienced mostly shady and humid conditions. A plastic roof placed above the containers prevented any rainwater or artificial spray from entering the traps. The volume of each preservative was re-measured every seven days. This was repeated until either the preservatives had completely evaporated or until 28 days had passed. A critical preservative volume of 100 ml was used, since below this volume specimens would not be fully submerged limiting their preservation. Note that the critical volume chosen was based on the dimensions of the pitfall trap containers used in this experiment and will thus change if different containers are used.

Results

Observations made during dissections are stated below under appropriate subheadings for each preservative. The relevant internal structures from freshly killed and dissected individuals are described first, followed by individual summaries of preservation success associated with each of the preservatives examined. The preservation category and the total number of days that the specimens were submerged are mentioned in brackets next to each preservative.

Freshly killed specimens

After removing the abdominal tergites the gut was exposed. A network of white trachea was readily visible along with elongated fat bodies which were either opaque white or yellow in colour. The entire gut could be carefully uncoiled and removed in one mass.

In females this exposed a single telotrophic ovary on the left side of the abdomen, as expected in the Scarabaeinae (Tyndale-Biscoe and Watson 1977). The ovary was made up of a number of oocytes, opaque white to yellowish orange in colour, in sequential stages of development. The oocytes were contained within a thin transparent ovariole wall. The largest most developed basal oocyte joined to the calyx and then

to the oviduct. The yellow body or corpus luteum forms on the base of the terminal oocyte and the calyx once egg laying commences (Tyndale-Biscoe 1978). A yellow body was not observed, possibly due to the individuals being nulliparous i.e. not laid eggs yet. Nonetheless, the ovariole base and calyx were clearly visible, thus allowing the detection of a yellow body to be made if it was present. The tip of the ovary consisted of the germarium which was slightly coiled and opaque white in colour.

In males the aedeagus along with the follicles were found lying on the inner bottom centre of the abdomen. The testicular follicles were round and opaque white in colour. The vas deferens was thick and opaque white in colour but was brighter than the follicles. The accessory glands were thin, elongate tubular in shape and opaque white in colour.

Brine (Poor – after seven days of submergence) (Figure 1). All the internal organs were dissolved and unpreserved. The gut was liquefied and the fat bodies were reduced to small white globules which sometimes formed large white coagulations. These white coagulations were not oocyte remnants as they were found in both male and female specimens. In females the ovary could not be detected. The male testes were partially preserved. They changed to a brown colour and became partially transparent revealing the seminiferous tubules, which had an opal like appearance. The follicles became supple and easily flaked apart. The vas deferens and accessory glands retained their original shape and size but changed to a dark brown and translucent colour respectively. The muscles and membranes connecting the thorax to the abdomen were also deteriorated since the two easily separated when the beetles were pinned for dissection. Due to the inadequate preservation of specimens in brine after the first seven days, no more individuals were dissected after this period, as further submergence would have not increased preservation success.

Saturated Borax solution (Poor – after seven days of submergence). Identical to brine.

Propylene glycol 33%, 50%, 75% (Moderate – after seven days of submergence). Since no difference in preservation between the three concentrations of propylene glycol could be found, they are summarised here under the same category. The gut was soft and spongy and was easily cut apart. Fat bodies were reduced to white grains or escaped as oil droplets once the abdominal tergites were removed. The ovariole wall, germarium and small oocytes were not preserved and only the larger oocytes were partially preserved. These oocytes were distorted in shape and flaked apart easily when handled. The ovariole base of the terminal oocyte and calyx could not be determined in order to check for an accumulation of yellow body. The male follicles were brown and partially transparent, revealing the seminiferous tubules, which had an opal like colour. The vas deferens and accessory glands retained their shape and colour but were easily distorted when handled. The thorax easily detached from the abdomen when individuals were pinned. Due to the inadequate preservation of specimens in the

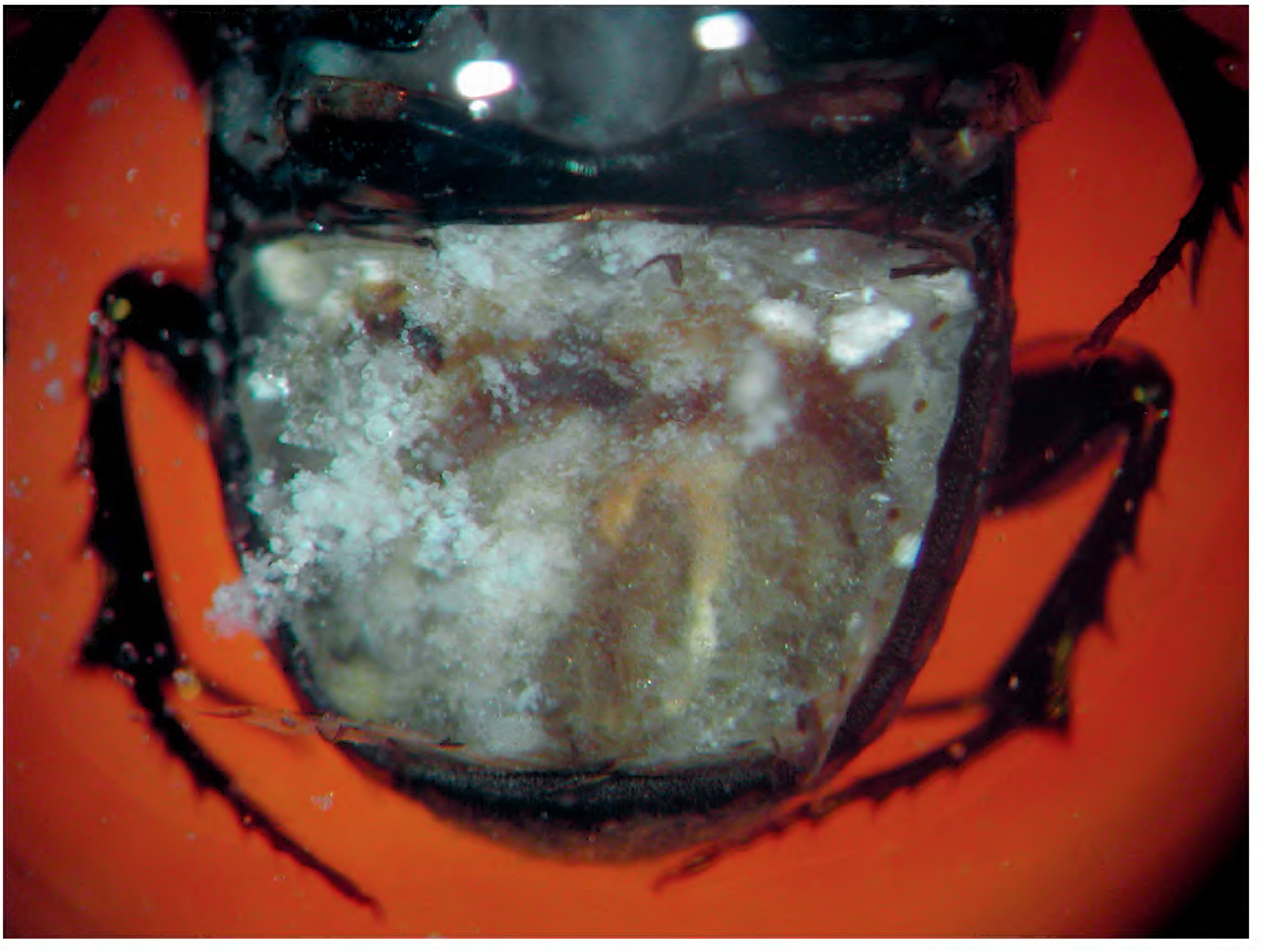

Figure 1. *L. militaris* (female) after seven days of submergence in brine showing the unpreserved internal organs.

three different concentrations of propylene glycol after the first seven days, no more individuals were dissected after this period, as further submergence would have not increased preservation success.

There was no notable difference in preservation between specimens kept in propylene glycol or transferred to 96% ethanol the day before they were dissected.

Ethylene glycol (Moderate – after seven days of submergence). The gut was partially deteriorated and the abdomen was filled with a brown liquid. The fat bodies were reduced in size and turned a light brown colour. The ovariole wall and germarium were not preserved and only the larger oocytes were partially preserved. In some individuals, but not all, these retained their shape. In both instances they were easily damaged and flaked apart once handled. The terminal oocyte was not connected to the calyx and oviduct so a yellow body category could not be determined. The male follicles were partially preserved. Only a few remained and these were a brown transparent colour and they were very supple. The vas deferens and accessory glands were only partially preserved. The thorax easily detached from the abdomen when individuals were pinned. Due to the inadequate preservation of specimens in ethylene glycol after the first seven days, no more individuals were dissected after this period, as further submergence would have not increased preservation success.

White vinegar (Moderate – after seven days of submergence). The gut was not preserved. The abdomen was filled with small white particles of deteriorated matter, possibly fat bodies. The ovariole wall and germarium were not preserved. The larger oocytes were partially preserved but did not retain their original shape and colour. They were easily damaged once handled and were not connected to the calyx and oviduct so a yellow body could not be determined. The male follicles, vas deferens and accessory glands retained their shape and colour but were easily distorted once handled. Due to the inadequate preservation of specimens in white vinegar after the first seven days, no more individuals were dissected after this period, as further submergence would have not increased preservation success.

FAACC (Good – after seven and up to 28 days of submergence). The gut, fat bodies and ovary were preserved retaining their original shape and colour. All the organs were fixed onto each other making it difficult to remove the gut and fat bodies that surrounded the ovary without breaking the oocytes. The ovariole wall was not preserved and the oocytes were only held in position because they were fixed to each other. The male follicles, vas deferens and accessory glands were preserved retaining their original shape and colour but they too were easily broken or snapped apart. No major difference to the above was observed after 28 days of submergence in FAACC.

There was no notable difference in preservation between specimens kept in FAACC or transferred to 96% ethanol the day before they were dissected.

4% PBF (Very good - after seven and up to 28 days of submergence) (Figure 2). The gut, fat bodies and reproductive organs were properly preserved and retained their original shape and colour. These structures could still be moved apart and had not fused together. The entire ovary was preserved with the germarium and all of the developing oocytes still intact within the thin ovariole wall. The terminal oocyte was still attached to the calyx and oviduct and so the area could be inspected for the presence of a yellow body. The ovary was also very rigid and could be moved around without the fear of breaking apart. Male follicles were no exception and they too were highly preserved along with the vas deferens and accessory glands. No major difference to the above was observed after 28 days of submergence.

There was no notable difference in preservation between specimens kept in 4% PBF or transferred to 96% ethanol the day before they were dissected.

96% Ethanol (Very Good – after seven and up to 28 days of submergence). Identical to 4% PBF

Evaporation rates

Since the preservative volumes were measured every seven days and not daily the evaporation rates are based on a linear rate of evaporation with time, therefore the predicted days to critical volume should be regarded as approximations. All of the

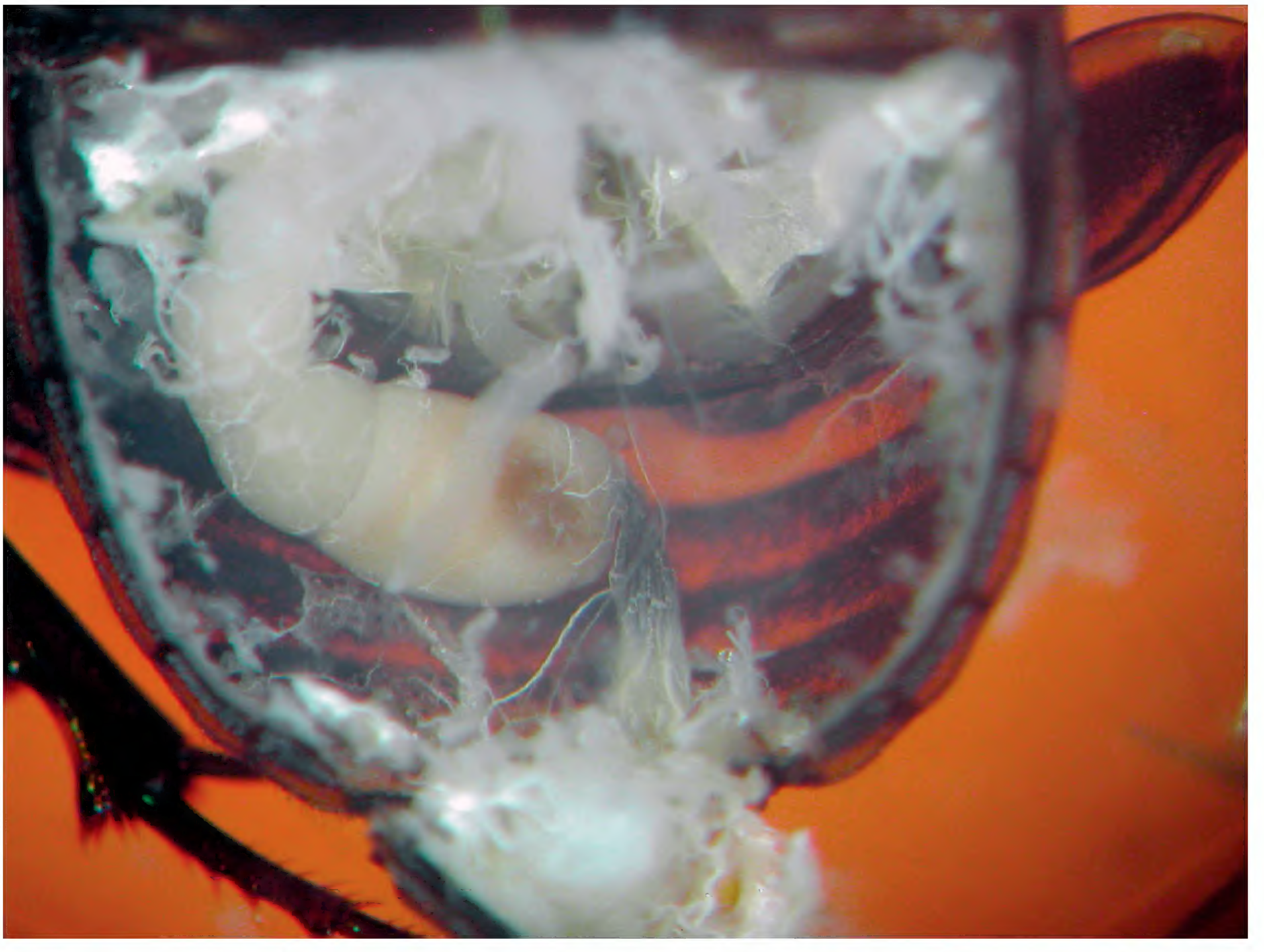

Figure 2. *L. militaris* (female) after 28 days of submergence in 4% PBF showing the well preserved ovary and oocytes.

preservatives evaporated at a slower rate within the shady and humid riparian vine thicket (Figure 3) in comparison to the sunny and dry, low open woodland (Figure 4). All of the preservatives in the vine thicket, except ethanol, remained above the critical volume after 28 days (Figure 3). However, in the open woodland FAACC, 4% PBF and the saturated Borax solution reached the critical volume in 18 days and brine in 21 days (Figure 4). Ethanol evaporated most readily out of all the preservatives, reaching critical volume within seven days in the vine thicket (Figure 3) and within the first five days in the open woodland (Figure 4). The hygroscopic property of ethylene glycol resulted in an increase in volume throughout the duration of the experiment at both sites (Figures 3 & 4). Propylene glycol was also observed to display hygroscopic activity especially at higher concentrations (Figure 3) or when the majority of water had evaporated from the lower concentrations (Figure 4).

Discussion

Only 4% PBF, 96% ethanol and FAACC adequately preserved all the dung beetle internal reproductive organs after 28 days of submergence (Table 1). Since 96% ethanol

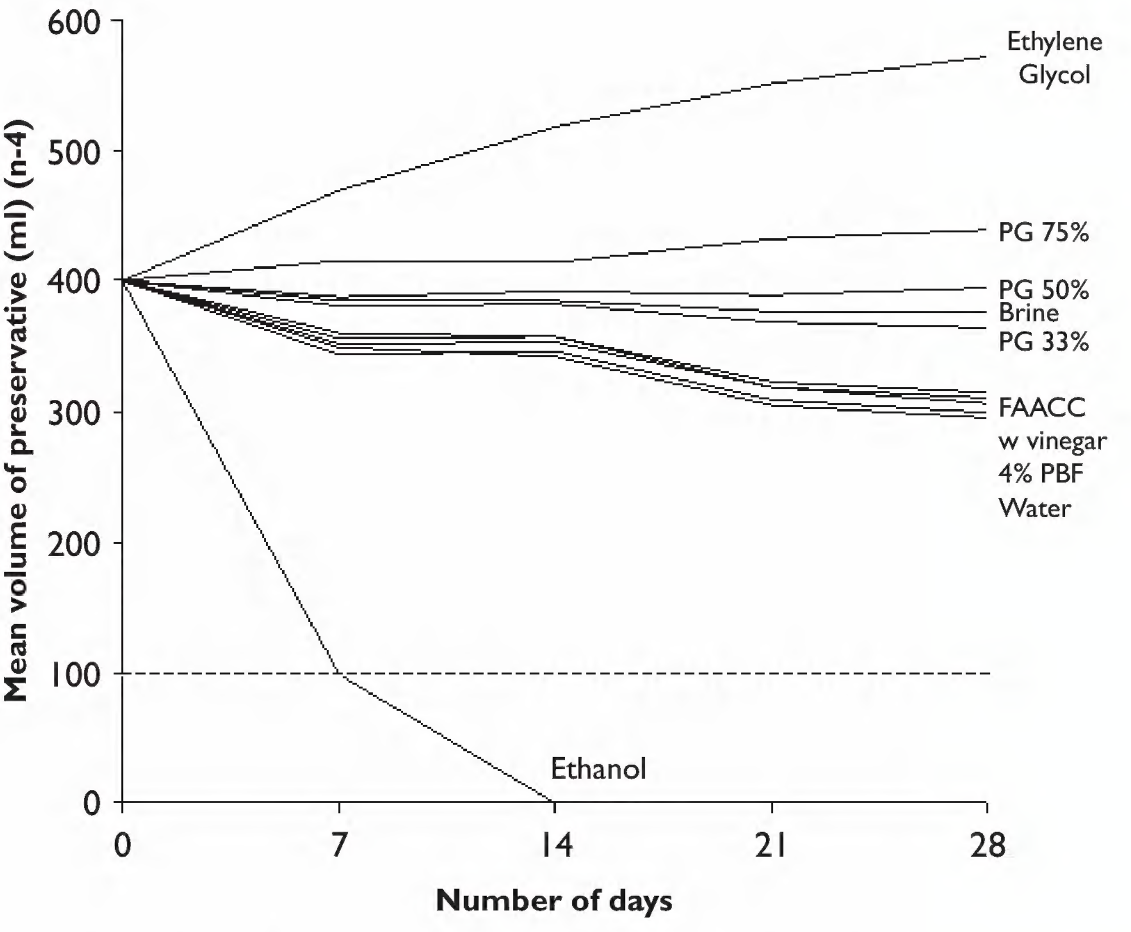

Figure 3. Evaporation rates of the eight preservatives in the riparian vine thicket environment. Water is also shown for comparison. The dotted line represents the critical volume. PG = propylene glycol, w vinegar = white vinegar.

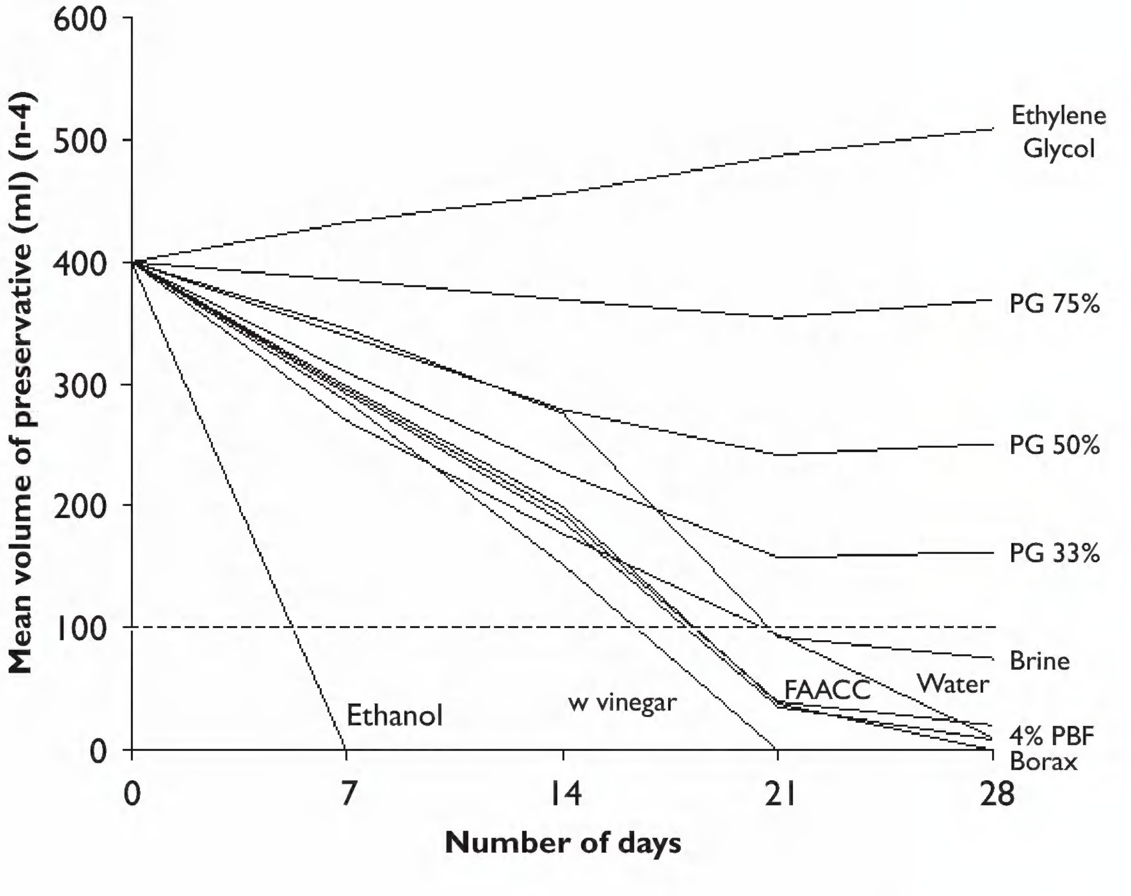

Figure 4. Evaporation rates of the eight preservatives in the low open woodland environment. Water is also shown for comparison. The dotted line represents the critical volume. PG = propylene glycol, w vinegar = white vinegar.

evaporates within a week in the field, and FAACC fused most of the internal organs together, 4% PBF is recommended as a suitable pitfall trap preservative for arthropod samples likely to remain in situ in the field for periods greater than one week and up to one month. Specimens remaining in 4% PBF for 54 days in the field have been dissected and found with well preserved reproductive organs (pers. obs). Since identical preservation occurred between beetles kept in 4% PBF or transferred to 96% ethanol, specimens can be removed from 4% PBF and stored in 96% ethanol until their subsequent dissection.

FAACC and formaldehyde are commonly used as gonad and cell fixatives, so it is unsurprising that they would preserve the internal organs of arthropods; similar results were also obtained with 96% ethanol. The low internal preservation strength of propylene glycol should be highlighted as it is a commonly used and often recom-

Table 1. Relative preservation of the dung beetle internal organs/structures by different preservatives in comparison to freshly killed specimens. All preservatives were tested in pitfall traps placed in the field, except for 96% ethanol which was tested in sealed vials in the lab.

	Preservative							
	Brine	Saturated borax solution	Propylene glycol 33%, 50%, 75%	Ethylene glycol	White vinegar	FAACC	4% PBF	99 % Ethanol
No. days beetles sub-merged in preservative	7	7	7	7	7	28	28	28
Internal organ/ structure								
Germarium	Not preserved	Not preserved	Not preserved	Not preserved	Not preserved	Good	Very good	Very good
Ovariole wall	Not preserved	Not preserved	Not preserved	Not preserved	Not preserved	Not preserved	Very good	Very good
Small oocytes	Not preserved	Not preserved	Not preserved	Not preserved	Not preserved	Good	Very good	Very good
Large oocytes	Not preserved	Not preserved	Poor	Moderate	Moderate	Good	Very good	Very good
Calyx	Not preserved	Not preserved	Not preserved	Not preserved	Not preserved	Good	Very good	Very good
Male follicles / testes	Moderate	Moderate	Moderate	Poor	Moderate	Good	Very good	Very good
Vas deferens	Moderate	Moderate	Moderate	Poor	Moderate	Good	Very good	Very good
Accessory glands	Poor	Poor	Moderate	Poor	Moderate	Good	Very good	Very good
Fat bodies	Poor	Poor	Poor	Moderate	Poor	Good	Very good	Very good
Gut	Not preserved	Not preserved	Moderate	Poor	Not preserved	Good	Very good	Very good
Overall preservation category	**Poor**	**Poor**	**Moderate**	**Moderate**	**Moderate**	**Good**	**Very good**	**Very good**

mended preservative for pitfall traps (Weeks and McIntyre 1997; Schmidt et al. 2006; Thomas 2008). The reduced preservation effect of brine and ethylene glycol was also reported for a study involving carabid beetles (Sasakawa 2007). Interestingly, Sasakawa (2007) was able to determine egg maturation, but not egg volume. The present study shows that this is not the case for dung beetles. These differences in egg preservation may be attributed to the differing egg maturing strategies/rates of the Carabidae and Scarabaeinae. Scarabaeinae dung beetles posses a single telotrophic ovary with one ovariole (Tyndale-Biscoe 1978). Eggs are matured sequentially and there can only be one mature egg possessing a chorion within the ovariole at any one time (Richards and Davies 1977). Thus, the remaining undeveloped (but developing) oocytes do not possess a chorion. On the other hand, Carabidae mature many eggs, which possess a chorion, that are stored in the oviducts (van Dijk and den Boer 1992). The chorion protects the egg against dehydration and physical damage (Hinton 1981). Thus, its presence may be responsible for the partial preservation of Carabid eggs in brine and ethylene glycol reported by Sasakawa (2007). This may also explain why the larger oocytes (more mature and likely to possess a chorion) of dung beetles submerged in ethylene glycol in this experiment were preserved in some individuals but not in others. Therefore, the lack of preservation of Scarabaeinae eggs by many pitfall trap solutions may be attributed to, but is not limited to, the late deposition of the protective chorion around the oocytes.

It should also be noted that most preservatives may act as either an attractant or repellent to certain species or groups of arthropods (Luff 1968; Greenslade and Greenslade 1971; Luff 1975; Weeks and McIntyre 1997; Pekar 2002; Schmidt et al. 2006). There does not seem to be any repulsive effect on dung beetles when using dung baited pitfall traps containing 4% PBF (pers. obs); it may be possible that the attractiveness of dung may mask or overpower any possible repulsive effects of formaldehyde and substantial numbers of dung beetles have been caught using this method (Aristophanous, unpublished data). However, since attractive or repulsive properties of formaldehyde on dung beetles were not tested for, in this or other studies, they remain unknown. Nonetheless, there are reported repellent effects of formaldehyde on Opiliones (Pekar 2002) and attractive effects on lycosid and theridiid spiders (Pekar 2002), carabid, staphylinid (Luff 1968; Pekar 2002), curculionid, and hydrophilid beetles (Luff 1968). In formaldehyde stored specimens, isolation of DNA is often difficult due to degradation and is thus not recommended for genetic studies (Gurdebeke and Maelfait 2002). Specialised techniques are required for the effective extraction of DNA and RNA sequences from formalin-fixed, paraffin embedded tissue (see Coombs et al. 1999).

The evaporation experiment revealed that the environment in which a pitfall trap is placed can affect the evaporation rate of the preservative (Figures 3, 4). Pitfall traps placed in an open woodland environment evaporated at a faster rate than the ones placed in the riparian vine thicket. 96% ethanol evaporated the fastest and should not be used for periods longer than a few days. Similar results were found by Schmidt et al. (2006) even when ethanol was mixed with glycerine or water i.e. lower con-

centrations of ethanol. Care should be taken if ethylene glycol is to be used, since its hygroscopic properties may lead to overspilling if it is left in the field for too long, alternatively lower concentrations may be used. Propylene glycol also showed hygroscopic properties at higher concentrations, thus a 50% concentration is recommended. FAACC and 4% PBF may be used for periods longer than four weeks in humid shady environments but in sunny drier environments their use will be limited to just below three weeks.

If long term trapping is to be undertaken with no desire to examine the internal organs then propylene glycol is recommended. Brine and borax solutions should not be used since within these preservatives crystallisation occurred on the exoskeleton of the dung beetle specimens. This will make identification of specimens difficult since many external features are hidden and any attempts in removing the crystals leads to the breaking of certain structures, especially the antennae and legs. Ethylene glycol should be avoided since it is toxic to wildlife (Hall 1991) and has similar preservative strength to propylene glycol (see Results and Table 1). Thus, propylene glycol is probably the easiest and safest to use since it is not toxic, is odourless and can remain in the field for prolonged periods. However, pitfall traps containing propylene glycol have been observed to be repeatedly disturbed by wild pigs and rats when placed in Australian rainforests (K. Staunton, pers. comm.) whereas pitfall traps containing 4% PBF in the same area where not disturbed (pers. obs.). Similarly, birds and mammals have been reported to consume ethylene glycol voluntarily even when water was available (Hall 1991). White vinegar is also a good candidate since it is cheap and easily obtainable. Norden et al. (2005) have successfully used rice vinegar to preserve mosquito ovaries, but only for short periods of time.

The results of this study emphasises the *a priori* need to determine the type and concentration of preservative that should be used. Clearly, this choice is dependent on the target species or group of species, their scientific use and storage (e.g. for dissection or genetics) and the habitat in which the pitfall traps will be placed.

Safety issues

Formaldehyde is classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC 2006). However, a recent comprehensive review of cancer has concluded that there was no excess risk for a number of cancers in industry workers and professionals exposed to formaldehyde (Bosetti et al. 2008). Nonetheless, all concentrations of formaldehyde should be treated with care, and it is highly recommended that appropriate precautions be taken when handling formaldehyde or any other hazardous chemicals e.g. use of latex gloves. Regardless of some views against the use of formaldehyde the author agrees with Pekar (2002) in that the majority of preservatives are toxic, but their use is sometimes unavoidable.

Protective caging and mound for pitfall traps

When placing pitfall traps containing formaldehyde in the field, two important factors should be taken into consideration: 1) mammals should be prevented from interfering with or gaining access to formaldehyde, and 2) formaldehyde should not leak or overspill the container into the surrounding soil.

To prevent mammal interference it is recommended that protective caging be placed around the trap (Newton and Peck 1975). This will also protect the pitfall containers from being chewed and destroyed by rats (a common problem in Australian rainforests). The easiest and most effective way to do this is to place a ring of aviary mesh wire (the mesh gaps should be big enough so as to allow the target organism to fit through) around the pitfall container. The ring of mesh wire is then pegged into the soil using pegs constructed from metal wire (similar to tent pegs). To protect the trap from precipitation a thin metal (zincal or aluminium) roof should be placed on-top of the mesh ring and attached in position by tying it with thin metal wire (Figure 5). To prevent water from entering the pitfall trap by surface runoff the containers should be placed 1/2 or 1/3 into the soil and a mound should be constructed around the containers (Figure 5). This will ensure that surface runoff will flow around the trap and not into it. Care must be taken to ensure that the lip of the container is flush with the soil. Two containers should be used so that the outer one will always remain in the soil and only the inner container removed when the trap is serviced. This makes clearing the trap easier and efficient as the soil is not disturbed every time the trap is visited.

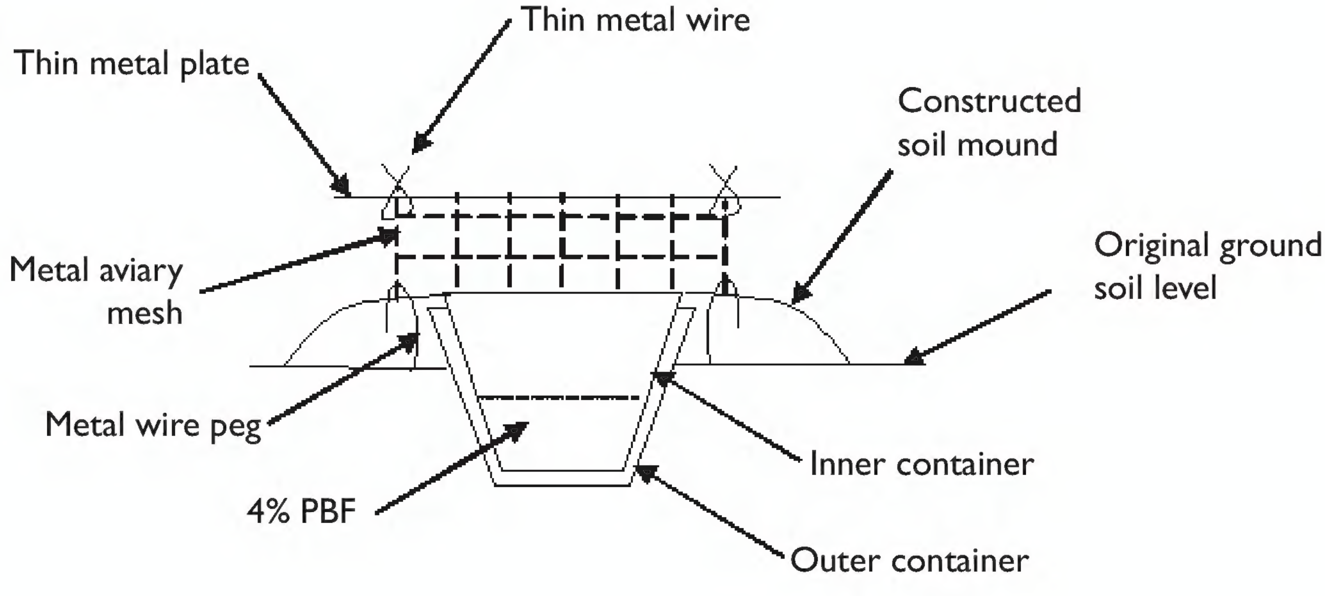

Figure 5. Pitfall trap with protective caging and cover placed on-top of a manually constructed soil mound so as to prevent interference from mammals and dilution and/or overspilling from precipitation and surface runoff.

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